Influence of sequential and mixed fermentations with non-*Saccharomyces* yeasts on the sensory profile of red wine

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Introduction

One of the main opportunities for using non-*Saccharomyces* yeasts is its great intraspecific variability in relation to the synthesis of secondary products of fermentation [1].

Mixed or sequential fermentations with non-*Saccharomyces* yeasts can potentiate colour stability by vitisins synthesis [2, 3, 4], or formation of vinylphenolic pyranoanthocyanins (VPAs) due to HCDC activity [5].

Regarding the aromatic profile, mixed and sequential fermentations allow increasing concentrations of some interesting compounds in red wine as ethyl lactate, 2,3-butanediol, 2-phenylethanol and 2-phenylethyl acetate [2, 4, 6]. Also achieving reductions in the content of higher alcohols is interesting [6].

T. delbrueckii species is characterized by its high purity fermentation and thus has low production of glycerol, acetaldehyde, acetic acid and ethyl acetate [7]. When used in sequential or mixed fermentations with *S. cerevisiae* allows correcting certain defects in wines as volatile acidity [8].

S. pombe species is highly appreciated in cold regions because of its ability to completely transform the malic acid of the must into ethanol thanks to maloalcoholic fermentation [9]. Moreover, their high ability to synthesize pyruvic acid (vitisin A precursor) and glycerol was recently described [3, 10].

The aim of this work is to evaluate the influence of *S. pombe* and *T. delbrueckii* species on the sensory quality of red wine when used in sequential and mixed fermentations with *S. cerevisiae*.

Results and Discussion

All fermentations ended with an alcoholic strength ranging from 13-13.4 % v/v. In general, the highest glycerol contents were produced in MF reaching ~7 g/l. (Table 1). SF kept slightly more tartaric acid in free form than MF, reaching mean values of up to 3.3 g/l (Table 1). MF in general, and particularly with *S. pombe*, was the one with highest concentrations of lactic acid produced during the alcoholic fermentation (~0.20 g/l) (Table 1). One of the major disadvantages of the use of *S. pombe* species is its greater acetic acid synthesis (0.5-0.7 g/l), what can be controlled in MF or SF with *S. cerevisiae*.

MF had higher values of monomeric, acetylated and coumarylated anthocyanins than SF, especially with *T. delbrueckii* strains, what is reflected in the higher total anthocyanins content (Figure 1). *S. pombe* strains in SF highlighted positively by its greater vitisins synthesis (Figure 2A), especially A type [3]. However, in MF both species produced similar amounts, probably due to the greater influence of the *S. cerevisiae* strain. As for VPAs, its synthesis was higher in MF. Maximum concentration was reached with *T. delbrueckii* species (~1.5 mg/l) (Figure 2B).

MF with *T. delbrueckii* species allowed increasing fruity aromas in the wine by synthesizing larger amounts of esters (Table 2). In turn, MF in general, produced significantly higher concentrations of polyols. On the other hand, SF enhanced herbaceous aromas (1-hexanol), but decreased the presence of total higher alcohols, primarily with *S. pombe* species. T. delbrueckii species in SF can produce significant amounts of 3-ethoxy-1-propanol (blackcurrant flavour).

As a general rule, for all the analysis performed, the biggest differences between species were registered in sequential fermentations. It certifies that this type of fermentation is suitable for enhancing the expression of the non-Saccharomyces yeasts' metabolic particularities, whereas with mixed fermentations greater uniformity is achieved

Conclusions

The use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* species in sequential and mixed fermentations with *Saccharomyces cerevisiae* **may improve the sensory profile of red wine by enhancing aromatic complexity and increasing colour stability**.

Materials and Methods

Three representative strains of each non-*Saccharomyces* species, *Schizosaccharomyces pombe* and *Torulaspora delbrueckii*, were assessed in sequential and mixed fermentations with a *Saccharomyces cerevisiae* strain. *S. cerevisiae* 7VA (HCDC+) (EnotecUPM, Madrid, Spain) was used as control strain in pure fermentation (PF) (Figure 3).

The fermentative assay was performed in triplicate at 23 ºC using a must of Syrah grapes (*Vitis vinifera* L.) with 220 g/l of initial sugar content and pH 3.5. In sequential fermentations (SF), 70 ml of must in 100 ml flasks were inoculated with 1 ml of each non-*Saccharomyces* strain, and after 7 days the second inoculation was perform with 1 ml of 7VA strain. On the other hand, mixed fermentations (MF) were coinoculated with 1 ml of non-*Saccharomyces* strain and 100 µl of 7VA strain (non-*Saccharomyces*:*Saccharomyces* ratio 10:1). Inoculum population was 10^6 cfu/ml.

Anthocyanins (HPLC-DAD), volatiles (GC-MS), organic acids (HPLC-UV), glycerol (HPLC-RI), alcoholic strength (HPLC-RI), residual sugars (HPLC-RI) and colour (spectrophotometry) were analyzed at the end of the fermentation.

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Table 1. Glycerol, tartaric acid and lactic acid content (g/l) of the wines obtained from mixed and sequential fermentations with *S. pombe* and *T. delbrueckii*. Mean ± SD (n=3)

	Mixed fermentation		Sequential fermentation		Pure fermentation
	S. pombe	*T. delbrueckii*	*S. pombe*	*T. delbrueckii*	*S. cerevisiae*
Glycerol	7.0 ± 0.0c	7.0 ± 0.1c	6.4 ± 0.1b	6.0 ± 0.2a	7.1 ± 0.0c
Tartaric acid	2.8 ± 0.0a	2.9 ± 0.1a	3.3 ± 0.1b	3.2 ± 0.1b	2.9 ± 0.1a
Lactic acid	0.21 ± 0.03d	0.17 ± 0.02bc	0.16 ± 0.01b	0.12 ± 0.00a	0.20 ± 0.00cd

Values in the same row with same letter are not significantly different (p <0.05).

Table 2. Concentration (mg/l) of different volatile compounds identified in the wines obtained from mixed and sequential fermentations with *S. pombe* and *T. delbrueckii*. Mean ± SD (n=3)

	Mixed fermentation		Sequential fermentation		Pure fermentation
	S. pombe	*T. delbrueckii*	*S. pombe*	*T. delbrueckii*	*S. cerevisiae*
Isoamyl acetate	8.49 ± 0.46bc	11.39 ± 0.34d	0.06 ± 0.05a	6.40 ± 3.00b	9.96 ± 1.36cd
Hexyl acetate	0.25 ± 0.04b	0.39 ± 0.02c	0.00 ± 0.00a	0.21 ± 0.06b	0.42 ± 0.05c
Ethyl caproate	0.58 ± 0.02b	0.64 ± 0.09bc	0.44 ± 0.06a	0.44 ± 0.13a	0.76 ± 0.04c
Ethyl caprylate	0.57 ± 0.04b	0.60 ± 0.04b	0.13 ± 0.02a	0.14 ± 0.03a	0.74 ± 0.08c
2,3-butanediol	13.51 ± 1.24c	14.61 ± 1.40c	5.15 ± 1.69a	7.35 ± 0.77b	13.98 ± 1.47c
1,2-propanediol	0.31 ± 0.02c	0.41 ± 0.04d	0.10 ± 0.05a	0.20 ± 0.04b	0.45 ± 0.09d
1-hexanol	1.53 ± 0.13a	1.34 ± 0.07a	3.35 ± 0.20c	2.33 ± 0.11b	1.49 ± 0.04a
3-ethoxy-1-propanol	0.18 ± 0.05a	0.74 ± 0.27a	0.01 ± 0.02a	7.65 ± 3.66b	0.22 ± 0.03a
Higher alcohols	131.32 ± 2.57c	115.70 ± 13.49b	59.49 ± 5.74a	116.26 ± 7.34b	133.39 ± 4.48c

Values in the same row with same letter are not significantly different (p <0.05).

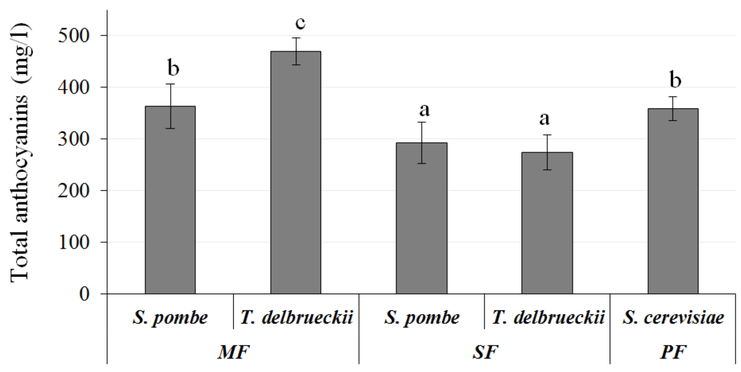

Figure 1.
Total anthocyanins content (mg/l) in mixed and sequential fermentations with *S. pombe* and *T. delbrueckii* strains. Mean ± SD (n=3). Bars with the same letter are not significantly different (p<0.05).

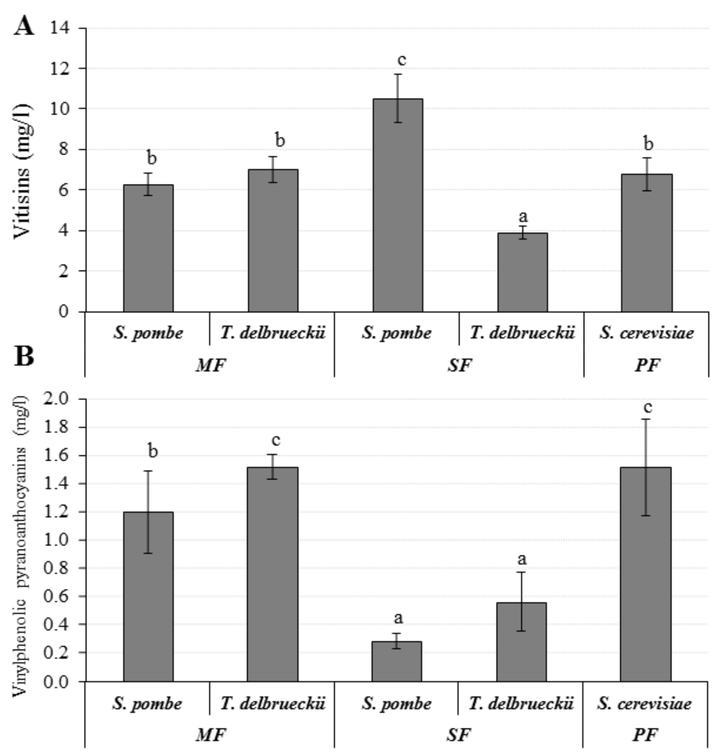

Figure 2. Vitisins and vinylphenolic pyranoanthocyanins content (mg/l) in mixed and sequential fermentations with *S. pombe* and *T. delbrueckii* strains. Mean ± SD (n=3). Bars with the same letter are not significantly different (p<0.05).

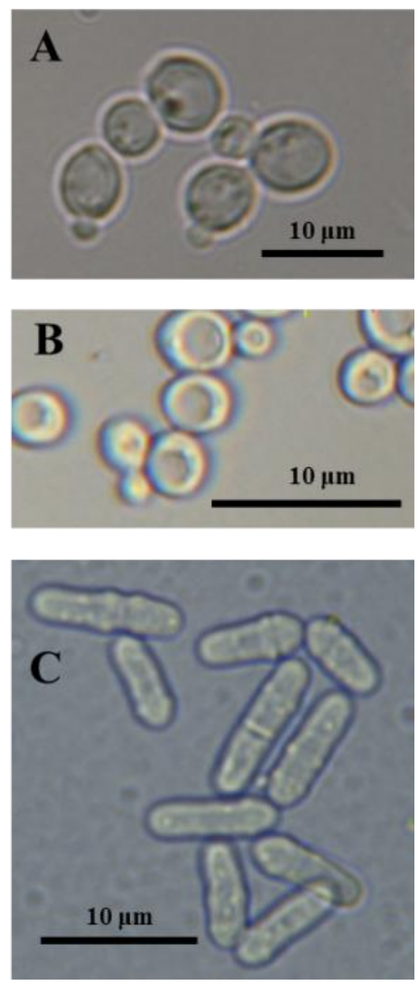

Figure 3.
Yeast species used in the fermentative assay. A: *S. cerevisiae*; B: *T. delbrueckii*; C: *S. pombe*.

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